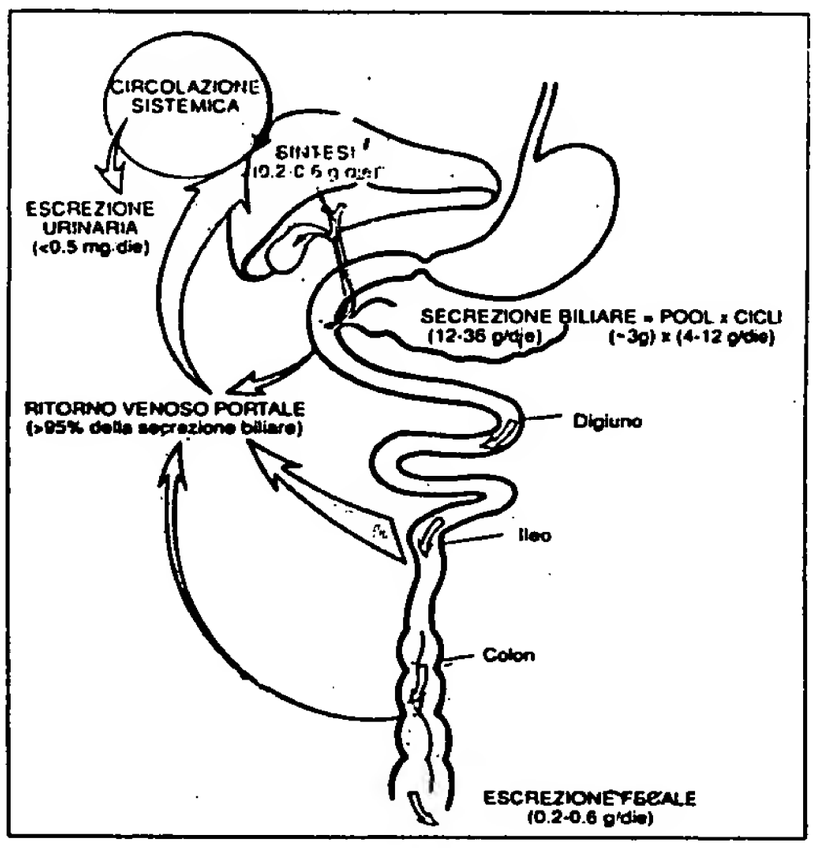
The present claims relate to gram-positive bacteria strains characterized by exhibiting:

(a) a 7α-dehydroxylase activity of less than 50%, and

(b) a bile acid deconjugation activity of less than 50%, and

belonging to a species selected from *Streptococcus thermophilus* and *Lactobacillus bulgaricus*, wherein said strain modifies bile acid metabolism.

As explained during the above-noted discussion with the Examiner, the bile acids which reach the gut exist in the conjugated and hydroxylated form. As such, these bile acids are not efficiently reabsorbed by the gut and are excreted with the feces. However, most of the hydroxylated, conjugated bile acids are ***de***hydroxylated and ***de***conjugated by the bacteria which populate the gut. In contrast to the conjugated, hydroxylated bile acids, the ***de***hydroxylated and ***de***conjugated bile acids are efficiently reabsorbed through the gut and enter the portal blood stream, then are taken up by hepatocytes, conjugated with glycine or taurine and resecreted into the bile, in the process known as enterohepatic circulation. This process is shown schematically in Figure 51-5 from M. C. Carey and S. C. Robins, "Production and secrection of bile acids," in Internal Medicine, Fourth Edition, J. H. Stern, Ed., Mosby-Year Book, Inc., St. Louis, 1994, which is given below:



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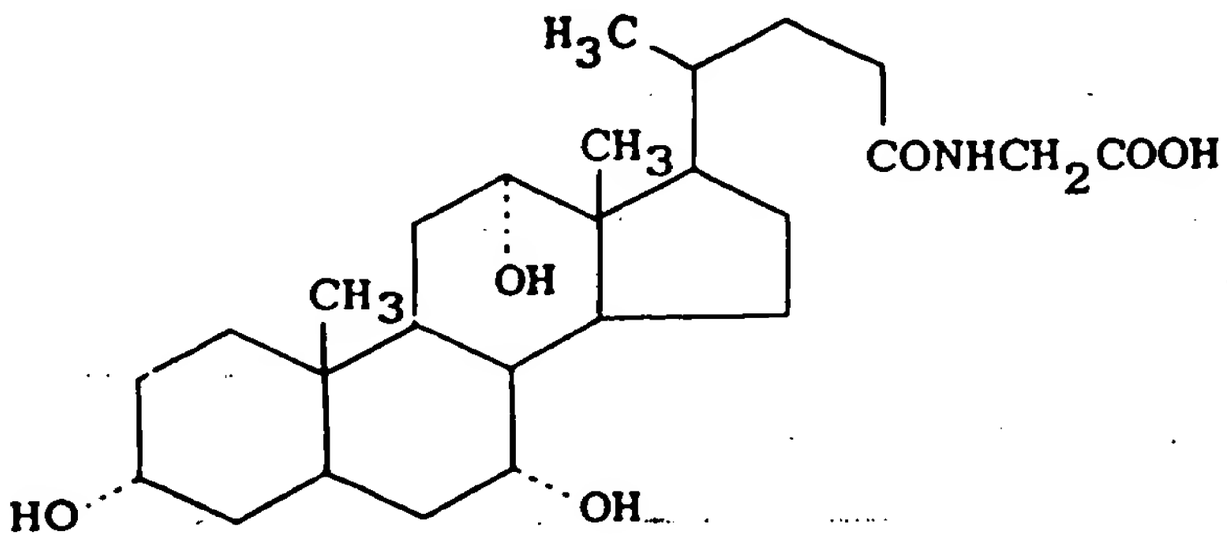
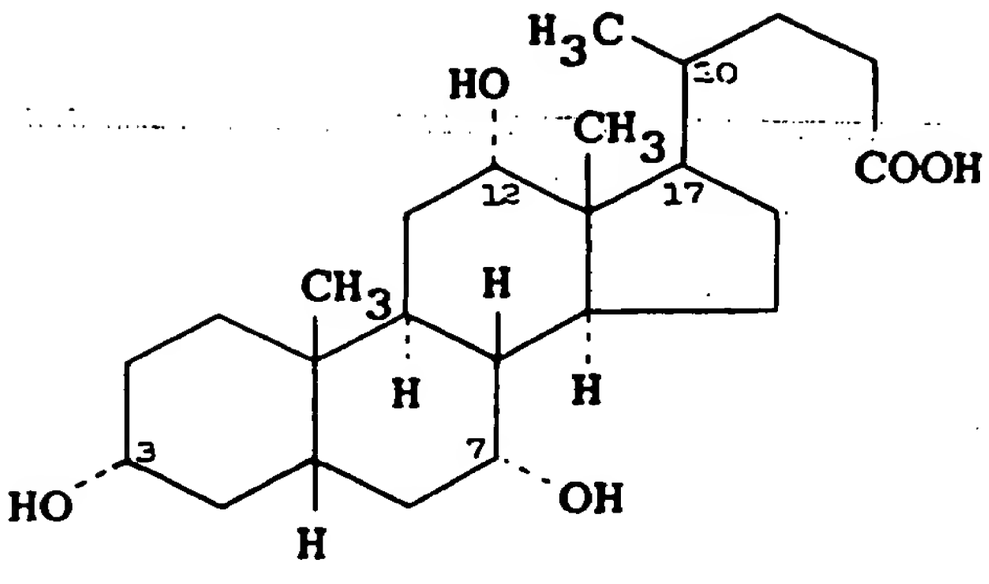
Under normal circumstances, the deconjugation and dehydroxylation of the bile acids in the gut, followed by reabsorption and transport to the liver causes no problems. However, certain states of disease in the liver may be caused or at least exacerbated by excessive amounts of ***de***hydroxylated and ***de***conjugated bile acids being transported back to the liver. These excessive bile acids can cause additional liver damage, as assessed by increased liver enzymes.

The present inventors have discovered that by colonizing the gut with bacteria which possess both a low dehydroxylation activity and a low deconjugation activity, it is possible to modify the bile acid metabolism such that less bile acid is reabsorbed in the gut and transported back to the liver. In this way, it is possible to restore the liver enzyme content of a person suffering from a condition mediated by bile acid metabolism to a more normal state.

As explained on pages 2 and 9-10, of the specification, in the context of the present invention, the term bile acid ***de***conjugation refers to the removal of a conjugated glycine or taurine group from cholic acid. For convenience, the conversion of glycocholic acid to cholic acid is shown below:

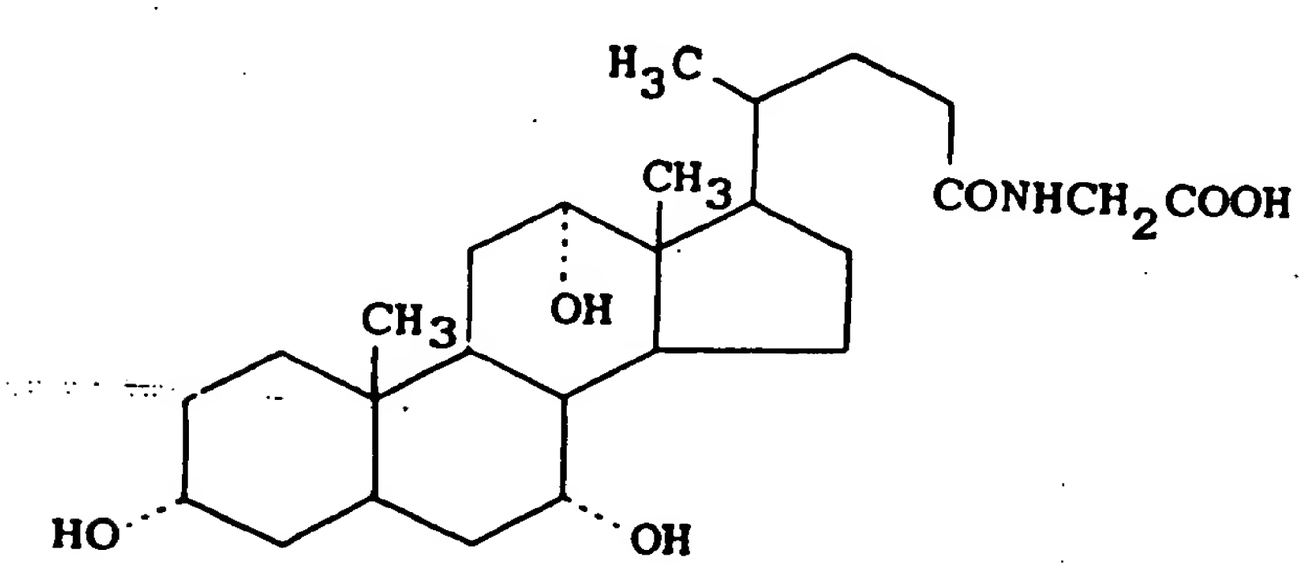


Glycocholic acid



Cholic acid

In contrast, as explained on pages 8 and 9, of the specification, the term "7α-dehydroxylase activity" as used in the present claims refers to the degree of 7α-dehydroxylation of either glycocholic acid or taurocholic acid. For convenience, the 7α-dehydroxylation of glycocholic acid is shown below:



Glycocholic acid

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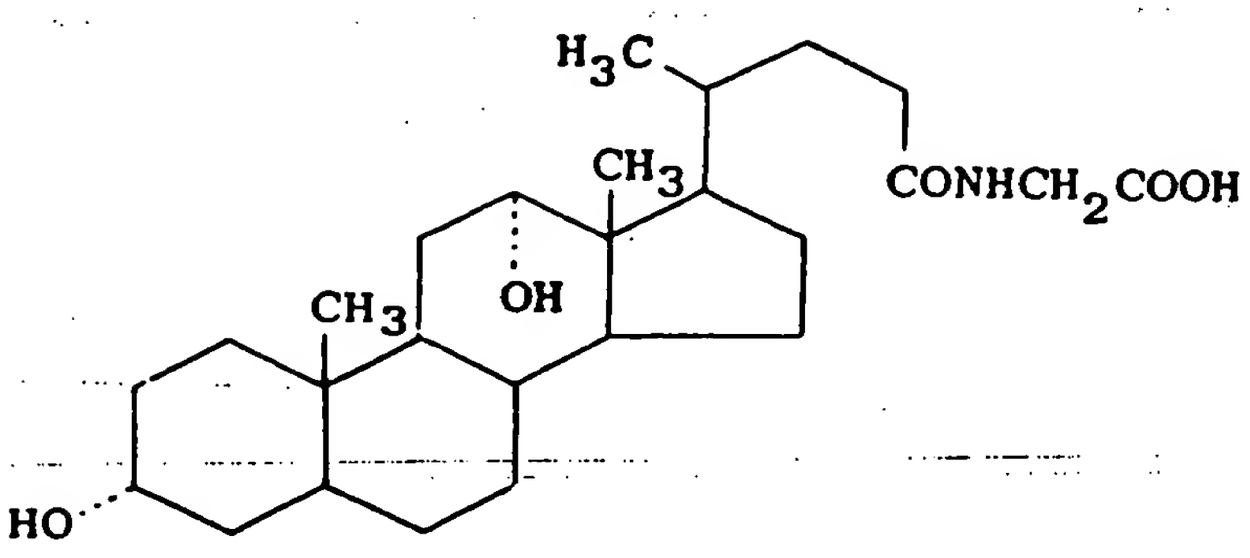

7α-Dehydroxylated glycocholic acid

As explained in the paragraph bridging pages 2 and 3 of the specification, deconjugation may precede 7α-dehydroxylation: "The unconjugated bile acids are therefore made available for .... dehydroxylation in positions 7α and 7β. See also, Salvioli et al: "Under normal conditions the deconjugation of bile salts occurs in the large bowel and perhaps in the terminal ileum; unconjugated BAs entering the large bowel are 7α-dehydroxylated by anerobic bacteria to yield secondary BAs."

Thus, the mere fact that a bacteria does not exhibit any 7α-dehydroxylation activity does not in any way imply that the bacteria also does not exhibit any deconjugation activity. Moreover, the mere absence of 7α-dehydroxylation does not imply "that there is no bile acid deconjugation."

It is important to recognize that the present claims are directed toward bacteria which have reduced activities for both deconjugation and dehydroxylation. Due to the large number (approximately 400) of different bacteria which populate the gut, administration of bacteria which has only a reduced deconjugation activity would not produce the desired result. It can

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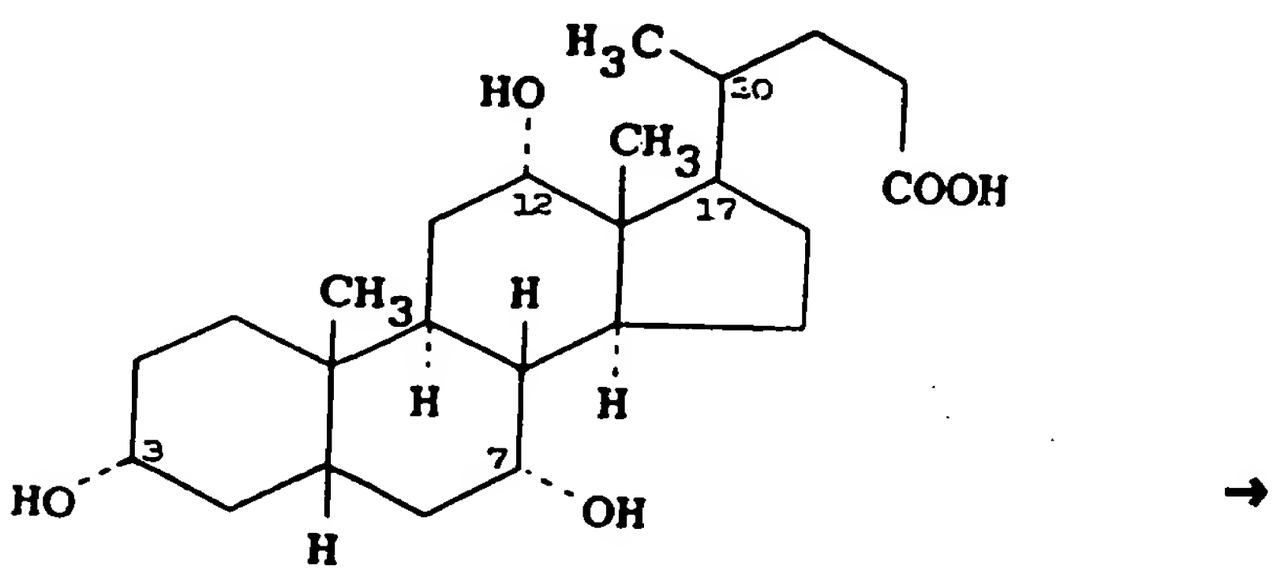
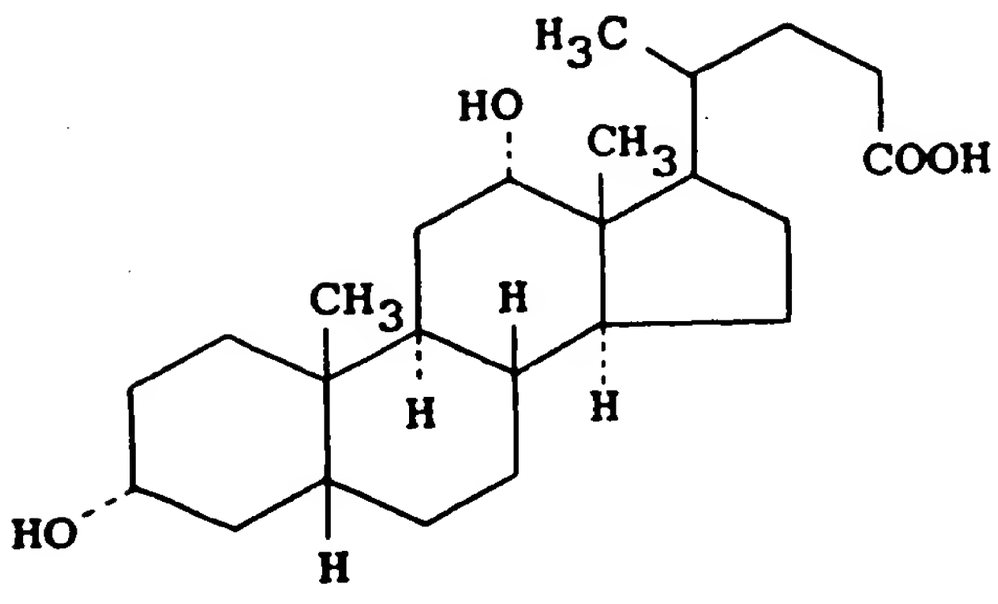
be easily seen that one of the other bacteria would simply pick up the slack by carrying out the deconjugation. Then the deconjugated bile acid could be dehydroxylated by the first bacteria, which has the reduced deconjugation activity.

The cited references contain no teaching which would suggest the presently claimed strains. Accordingly, these references cannot affect the patentability of the present claims.

The rejection of Claims 37-40 under 35 U.S.C. §102(b) in view of Takahashi et al 1994 in light of Salvioli et al is respectfully traversed. Takahashi et al reports the results of experiments to assess the ability of certain bacteria to 7α-dehydroxylate cholic acid. Thus, at the outset, it should be noted that the "7α-dehydroxylase activity" reported in Takahashi et al is not the same as that referred to in the present claims. As explained above, as used in the present claims, the term "7α-dehydroxylase activity" refers to the conversion of either glycocholic acid or taurocholic acid to a 7α-dehydroxylated product, while that term, as used in Takahashi et al, refers to the following reaction:



Cholic acid



Deoxycholic acid

In fact, Takahashi et al reports no results for any experiments using either glycocholic acid or taurocholic acid. Thus, this reference is completely silent with regard to the ability of any of the tested bacteria to deconjugate either glycocholic acid or taurocholic acid. Moreover, as explained above, since 7α-dehydroxylation typically follows (rather than precedes) deconjugation, the mere fact that a bacterium does not exhibit any 7α-dehydroxylase activity says nothing about the ability of that bacteria to deconjugate either glycocholic acid or taurocholic acid.

In summary, Takahashi et al is completely silent with regard to the 7α-dehydroxylation of either glycocholic acid or taurocholic acid. Thus, this reference does not disclose any bacteria having "a 7α-dehydroxylase activity of less than 50%" as recited by the present claims. Moreover, since Takahashi et al does not report any experiments at all with either glycocholic acid or taurocholic acid, there is nothing which would lead the skilled artisan to believe that the bacteria of Takahashi et al have the "bile acid deconjugation activity" recited in the present claims. For all of these reasons, the rejection is improper and

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should be withdrawn.

The rejection of Claims 37-44 under 35 U.S.C. §102(b, d, or e) or, in the alternative, under 35 U.S.C. §103 in view of Saito et al (U.S. Patent No. 5,516,684 or EP 0 671 468) in light of Salvioli et al is respectfully traversed. Saito et al simply teaches that the bacteria of the genus Lactobacillus do not exhibit deconjugation of bile acids. However, contrary to the position taken in the Official Action, this does not imply that these bacteria also possess a reduced 7α-dehydroxylase activity.

In fact, as reported in Lewis et al, Arch. Intern. Med., vol. 130, pp.545-49 (1972) (cited by the Examiner):

> In the human bowel, **7-dehydroxylation may occur without deconjugation**. Hepner et al fed normal subjects radioactively labeled glycocholic acid and showed that labelling appeared in the feces in deoxycholylglycine. This suggested that **7-dehydroxylation of cholyl glycine occurred without deconjugation**, since ingested labeled glycine is not incorporated into bile acid glycine.

(See in particular page 547, right hand column).

In fact, the prior art cited by the Examiner clearly supports the fact that bacteria may exhibit 7α-dehydroxylation activity without deconjugating the bile acid. As explained above, Takahashi et al reports the results of experiments designed to assess the ability of bacteria to 7α-dehydroxylate cholic acid to deoxycholic acid. Thus, this reference employs a a experimental design in which the 7α-dehydroxylation reaction occurred without any deconjugation!

Therefore, it is clear that the two activities (deconjugation and 7α-dehydroxylase of bile acids) must be considered as completely separate activities, which are independent from each other. These activities are possessed by the bacteria separately and, if a strain possesses one of these activities, it is not obvious or to be expected that it possesses also the other

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activity (see Lewis et al cited above).

It is further pointed out that nowhere in text of Saito et al is it mentioned or even suggested that the bacteria of the genus Lactobacillus exhibit a 7α-dehydroxylation activity. Hence, in the light of Saito et al and in the light of Lewis et al mentioned above, it was not to be expected and it was surprising to find some strains which exhibit both the activities simultaneously, as do the strains of the species *Streptococcus thermophilus* and *Lactobacillus bulgaricus* presently claimed by the Applicants.

This is also confirmed by a comparison between Table I and Table II, wherein it is demonstrated that many strains possess only the deconjugation activity or only the 7α-dehydroxylase activity.

Applicants again note that Salvioli et al is not relevant because the species *Streptococcus faecium* is not claimed in the present claims. In any case, this reference does not mention a deconjugation activity and only teaches that the strain *Streptococcus faecium* exhibits a 7α-dehydroxylase activity by means of an indirect mechanism, that is by changing the intestinal milieu and acting against Clostridia and other intestinal microorganisms (see page 80, column 1, line 3 from top; page 87, column 1, line 4 from bottom). Moreover, it is pointed out that the strains *Streptococcus thermophilus* and *Lactobacillus bulgaricus* presently claimed are aerobic strains, while the passage at page 80, column 1 cited in the Official Action relates to anaerobic bacteria.

In summary, none of the cited references disclose or suggest the presently claimed bacteria strains.

In further support of the unobvious nature of the presently claimed strains, Applicants cite the unexpected advantages afforded by the presently claimed strains. In particular, the Examiner's attention is first directed toward Example 2 given on pages 23-25, of the

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specification. In Example 2 , three volunteers were fed bacteria according to the present claims for 28 days. The composition of the bile of each volunteer before and after the treatment is set forth in Table III on page 24. As stated on page 25, this experiment shows that the "bacteria can reduce the detergency property and therefore the cytolytic activity of the bile acids."

The Examiner's attention is also directed toward Example 3 given on pages 25-27 of the specification. In Example 3, fourteen patients suffering from chronic hepatitis were treated with a mixture of the presently claimed bacteria for 28 days. The condition of each patient as indicated by the concentration of certain liver enzymes before and after the treatment is reported in Table IV on page 27, which shows that the enzyme concentrations were dramatically reduced in nearly every patient.

There is no teaching in any of the cited references which would suggest these dramatic results.

For all of these reasons, the rejection is improper and should be withdrawn.

The objection to Claims 38-44 and the rejection of Claims 37-44 under 35 U.S.C. §101 have been obviated by appropriate amendment. As the Examiner will note the claims have been amended such that they are free of the criticisms outlined on pages 2 and 3 of the Official Action. Accordingly, the objection and rejection are no longer tenable and should be withdrawn.

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Applicants submit that the application is now in condition for allowance, and early notification of such action is earnestly solicited.

Respectfully submitted,

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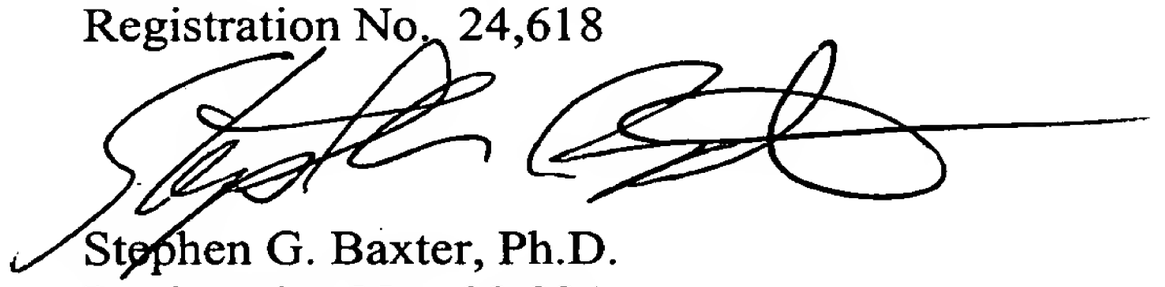

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